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(54) PROCESS FOR PREPARING ADSORBED VACCINES

(71) We, INSTITUT PASTEUR, a Public Service Establishment, of 25 due du Docteur Roux, Paris, France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to an improved process for the preparation of adsorbed vaccines.

The advantages of adsorbed vaccines are known. The concentrated form in which they may be present provides the advantage of only requiring a small space for their storage, whilst it is possible to prepare, at any moment, from this form large quantities of vaccines in very little time; this is particularly valuable in the event of epidemics or other immediate necessity; the adsorbed preparations which remain stable for several years enable the control of their dilution products to be considerably reduced, the necessary titrations having been carried out on the concentrated products. For these reasons the adsorbed vaccines have formed the subject of various works, not all of which have led to satisfactory results; in fact, the majority of adsorbants which have been proposed have the drawback that they are irritants, that they badly adsorb the antigens or that they are difficultly adaptable to the industrial scale. Some progress has been made with the use of a calcium phosphate gel prepared by the known method of Tiselius, that is to say a gel formed of brushite the phosphate whereof is in the form of

$$CaHPO_4 . 2H_2O.$$

However, even in this form the gel does not absorb sufficient antigens in all interesting cases and quite often its harmlessness leaves much to be desired. A further improvement has ameliorated the vaccines adsorbed on calcium phosphate gel, i.e. the one which forms the subject of Belgian Patent No. 724,141 and which consists in precipitating the phosphate within a medium containing the antigen.

The present invention desirably brings about an improvement in the preparation of a calcium phosphate gel which results in an appreciable improvement over the adsorbed vaccines hitherto prepared. The present invention enables very strongly adsorbing gels to be obtained. The injection of such adsorbing gels, prepared according to the invention, reduces or eliminates the possibility of irritations, or complications. Moreover, the novel calcium phosphate gel according to the invention can be utilised for adsorption after having been prepared and in order to preserve its good qualities it need no longer be formed within a medium containing the antigen.

According to the present invention we provide a process for the preparation of a vaccine adsorbed on a gel of calcium phosphate by contacting an antigen with an aqueous gel obtained by the reaction between bisodium phosphate and calcium chloride, wherein a solution of calcium chloride in water is poured within less than 3 minutes into an equimolar amount of an aqueous solution of stirred bisodium phosphate, and wherein, with continuing stirring, the pH of the mixture is adjusted, after the end of the calcium chloride addition, to a value of 6.8 to 7.2, the gel obtained being subsequently cleansed by sedimentation and decantation.

The invention results from the surprising observation that the very rapid addition of the calcium salt leads to a calcium phosphate which differs from brushite and from dibasic phosphates which form when an aqueous solution of calcium chloride is gradually added, with stirring, to a solution of bisodium phosphate. In fact, the phosphate which constitutes the gel according to the invention has a chemical composition nearer to tricalcium phosphate $Ca_3(PO_4)_2$ while the phosphates which have hitherto been used for vaccine adsorption had the composition of brushite

$$CaHPO_4 . 2H_2O$$

or near to this composition. When the mixing is effected at the elevated rate envisaged by the invention a part of the phosphorus

ions remains in solution, the precipitate being richer in calcium than the theory would indicate, even if the proportions of the starting materials, bisodium phosphate and calcium chloride, are equimolar.

The molarity of the solutions used according to the invention may advantageously be between 0.01 and 0.5, preferred values being from 0.05 to 0.1. Within these limits the aqueous solution of calcium chloride can have a concentration differing from that of the bisodium phosphate but its volume is such that in the final mixture there is substantially 1 mol of the one per 1 mol of the other of the reagents.

Although the duration of adding the calcium salt to the bisodium phosphate may be up to three minutes, it is preferable for it not to exceed one minute. Excellent results obtained when one works with such volumes that it is possible to pour the aqueous solution of $CaCl_2$ instantly into that of bisodium phosphate. Thus, with quantities of the order of 50 l. it is possible to perform the mixing in a time of 2 to 30 seconds.

One practical way of operating consists in placing the solution of bisodium phosphate into a container or pan equipped with stirring means and to rapidly empty into that container the contents of a reservoir containing the corresponding volume of calcium chloride; during this operation the stirring means are running and stirring is continued after introduction of $CaCl_2$ has been terminated. Especially favourable results are obtained when using a vibrator but the agitation could also be effected by means of other devices such as a vane stirrer or a turbo-mixer.

A further important characteristic of the new process resides in an adjustment of the pH of the mixture obtained, as soon as possible after addition of the calcium chloride. At this moment, the pH is brought to a value equal to or very near 7, more particularly 6.8 to 7.2, the range 7 to 7.2 being preferred. This adjustment can be made with the aid of a solution of sodium hydroxide the preferred concentration whereof is between 0.1 N and 1 N.

As mentioned above, adjustment of the pH should follow as quickly as possible the introduction of $CaCl_2$ into the aqueous solution of bisodium phosphate; in practice it is desirable for this adjustment to take place in less than 10 minutes after preparation of the mixture

To obtain better results it is preferred to proceed as follows:—

For the adjustment of the pH to be immediate, i.e. it should take place during the 30 to 90 seconds following preparation of the mixture. The above described operations are performed at temperatures which may vary between 5° and 40°C; in practice they can be performed at ambient temperature.

The mixture is then left to stand until the clear super-natant volume reaches 80 to 90% of the total volume. This standing may be at ordinary temperature or in a freezer and it generally lasts 6 to 18 hours. The supernatant liquid is then decanted.

Examination of the gel obtained and of the separated liquid shows that all of the initial calcium is to be found in the phosphate precipitate while a portion of the PO_4 ions remain in solution in the clear liquid accompanying the gel. This latter characteristic is an indication of a precipitate conforming to the present invention which leads to the novel phosphate gel close to tricalcium phosphate possessing the improved properties discussed above.

After decantation of the clear liquid an 0.4% to 0.9% solution of sodium chloride in water is added to the gel, the volume of this solution being of the same order or equal to that of the decanted liquid. The whole is mixed with the aid of a vibrator and again allowed to stand. The fresh layer of clear supernatant liquid is in turn removed and again replaced by a similar solution of sodium chloride. This operation is optionally repeated a third time (or a total of two to four times altogether) to complete washing of the gel.

Purification of the gel of calcium phosphate is of great importance. It has, in fact, been observed that the phosphorus ions present in the solution inhibit adsorption of certain antigens. According to the present invention adsorption is ameliorated by elimination of these ions. This elimination is advantageously realised by the above described washing of the gel with a saline solution; preferably the washing is effected by dilution with an aqueous solution of NaCl followed by sedimentation; the operation is repeated several times if necessary. It is particularly recommended to use a solution of NaCl containing 4 to 9 g of this salt per litre; in fact, the final dilution of the vaccine with such a solution is favourable to the action of the vaccine.

The thus washed final gel generally contains an amount of phosphate such that its phosphorus content per litre is from 0.3 to 1.5 g and preferably 0.7 to 0.85 g. The overall chemical analysis carried out leads, for the phosphate of the gel, to a composition comprised between those of dicalcium and tricalcium phosphates; in fact one finds molar ratios Ca/PO_4 varying between 1.20 and 1.45 and mainly between 1.25 and 1.38 which corresponds to weight ratios Ca/P of 1.55 to 1.90 and mainly 1.62 to 1.85; the ratio Ca/P by weight of brushite,

$$CaHPO_4 . 2H_2O$$

being 1.29 and that of $Ca_3(PO_4)_2$ 1.98 it is apparent that the overall composition of the

phosphate of the gel according to the invention lies in the neighbourhood of

$$CaHPO_4 . Ca_3(PO_4)_2$$

wherein the Ca/P ratio is 1.72 (molar ratio $Ca/PO_4 = 1.33$).

The gel obtained may be sterilised in an autoclave at 120°C for one hour whereafter the pH is adjusted to 6.8 to 7.2 and preferably in the range of 7. to 7.2 so as to correct the lowering of the pH towards approximately 6.5 which occurs during sterilisation. It is now ready to serve as adsorbent for various antigens in a manner known per se. It is to be well understood that the term antigen in the present description includes all kinds of substances of microbial secretion, for example the anatoxines, as well as whole micro-organisms such as bacteria, viruses or others, or certain of their fractions.

It has been noticed that with the precipitated phosphates according to the invention it is possible to make the gel adsorb one or several different antigens even when this gel has already previously adsorbed another antigen. Thus the present invention comprises also the preparation of mixed vaccines by addition of a calcium phosphate gel which has adsorbed a specific antigen to a solution containing one or several other antigens.

One or several kinds of antigens may be present in the precipitation medium, more particularly in the starting solution of bisodium phosphate; thus a gel containing adsorbed antigens is directly obtained. After separation of the mother liquors and washings this gel is capable of additionally adsorbing other antigens; it is thus possible to obtain mixed adsorbed vaccines by successive operations the first of which is an adsorption *in situ* during the precipitation and the second and adsorption of already formed gel.

The invention includes the aforesaid process wherein one or a plurality of antigens in aqueous medium are first adsorbed by mixing with the gel of calcium phosphate, the resultant gel is cleansed by sedimentation, separation of the supernatant liquid, and replacement of the latter by a saline solution, once or several times, and in that when one or a plurality of antigens of a different nature, also in aqueous medium, are adsorbed by the same gel which already contains the first antigen or antigens, and the gel so obtained is washed.

The present invention will now be described with reference to the following examples.

EXAMPLE 1

Preparation of adsorbed inactivated poliovirus vaccines.

To 50 l. of an 0.07 M solution of bisodium phosphate, continually stirred by means of a vibrator, 50 l. of an 0.07 M solution of calcium chloride are added in 28 seconds; the pH of the mixture is adjusted to 7.1 with a solution of normal sodium carbonate, following the addition of the $CaCl_2$. The 100 l. of calcium phosphate gel thus obtained are left to stand until clearing of 85 l. of liquid; the clear supernatant liquid is separated by siphoning and replaced by 85 l. of sterile aqueous solution containing 4 g/l. of NaCl.

A second washing is then effected by decantation of the saline liquid and a further addition of 85 l. of 4 g NaCl per litre of water. The 100 l. of suspension obtained are sterilised at 120°C whereafter the pH is adjusted to 7; the product is left standing and then 85 l. of supernatant liquid are decanted.

To 15 l. of gel remaining at the bottom of the vessel 100 l. of an aqueous solution of polyiomyelitis vaccine are added; the mixture is stirred for 30 minutes with the aid of a vibrator; the 115 l. of product are then left standing until formation of 100 l. of clear supernatant liquid, the latter is next separated which leaves 15 l. of suspension of calcium phosphate gel having adsorbed the polyiomyelitis vaccine.

To the latter volume a further portion of 100 l. of vaccine is added as on the first occasion. After vibration for 40 minutes a further decantation of 100 l. liquid is performed.

To 15 l. of suspension of phosphate gel charged with antigens of polyiomyelitis remaining after the decantation a third portion of 100 l. of the same polyiomyelitis vaccine is added as previously. After further vibration for 45 minutes the product is left to stand and 100 l. of supernatant liquid are decanted, leaving 15 l. of gel suspension.

The latter thus results from 3 successive adsorptions of vaccine and constitutes a product with a high concentration of the latter. It should be noted that in the course of the above mentioned decantations the supernatant liquid was always inactive which proves that the vaccine had been completely adsorbed.

To the 15 l. of suspension finally obtained a solution of 85 l. of 4 g/l NaCl containing 25 p.p.m. hyamine is added. The mixture is again agitated by means of a vibrator; after standing 85 l. of liquid are decanted, to the 15 l. of finally obtained gel 85 l. of a 4 g/l NaCl solution containing 25 p.p.m. hyamine as preservative are added. The resultant vaccine is three times as concentrated as the initial vaccine and it is free of undesirable constituents.

As to the contents of calcium phosphate, this corresponds in the 100 l. of final product to 0.897 g/l. of elementary phosphorus and 1.5 g/l. of calcium. Thus the weight ratio

Ca/P is 1.68 (atomic ratio 1.292) in the final gel, while at the beginning 1 mol $CaCl_2$ has been used per 1 of Na_2HPO_4, i.e. weight ratio Ca/P 1.29; in fact while all the Ca has precipitated a part of the phosphorus ion remained in solution in the eliminated supernatant; 0.31 g of P per litre of clear liquid of the first decantation were actually found.

EXAMPLE 2

Preparation of an anti-rabies vaccine.

The operations are those of Example 1 but the poliomyelitis vaccine is replaced by a suspension of rabies virus obtained by culture on sheep's brain. First a homogeneous suspension of brain containing the virus is prepared by grinding or by any other method. After the last decantation of supernatant liquid the latter is replaced by a solution of 9 g/l. of sodium chloride.

Simliar preparations are obtained by culture of the virus on the brain of suckling mice or by cellular culture.

EXAMPLE 3

Preparation of a mixed vaccine.

First a suspension of calcium phosphate is prepared as in Example 1 in situ in a solution containing diphtheria and tetanus vaccines. The 100 l. of suspension thus obtained are left to stand until 4/5 of the liquid has separated whereafter the clear supernatant liquid is removed. To the remaining suspension of gel 100 l. of inactivated polio-virus vaccine solution are added and the whole is stirred for 30 minutes with the aid of a vibrator. After standing the supernatant liquid is again separated. Then a further volume of 100 l. of the solution of inactivated polio-virus vaccine is added, stirring is renewed for half an hour and one decants so as to finally have no more than 20 l. of product (this operation may be repeated several times), which is then completed with 80 l. of 4 g/l NaCl containing 25 p.p.m. hyamine as preservative.

EXAMPLE 4

Preparation of a mixed vaccine.

Following the procedure of Example 1 there are prepared 25 l. of diphtheria vaccine adsorbed on calcium phosphate, having an antigen concentration 4 times greater than the final product, 25 l. of tetanus vaccine of the same kind and concentration and 25 l. of similar poliomyelitis vaccine. The three volumes of 25 l. are mixed and 25 l. of calcium phosphate gel obtained as in Example 1 are added to the mixture. The result is a composite diphtheria - tetanus - poliomyelitis vaccine adsorbed on calcium phosphate gel.

In another similar operation the 25 l. of phosphate gel are replaced by 25 l. of pertussis vaccine adsorbed on calcium phosphate, having an antigen concentration 4 times greater than the final product; a quadruple composite vaccine is thus obtained.

EXAMPLE 5

Mixture with a lyophilised vaccine.

In this preparation 50 l. of gel suspension which has adsorbed diphtheria and tetanus vaccines prepared according to one of the preceding examples are employed for absorbing a lyophilised live attenuated measles vaccine. After absorption of the lyophilised vaccine the mixture is ready to be injected.

EXAMPLE 6

Improving an adsorbed tetanus vaccine.

A tetanus vaccine is prepared by adsorption by means of a calcium phosphate gel formed *in situ* as in Example 1. To improve the quality, and avoid reactions of intolerance to which they often give rise, 100 l. of vaccine is allowed to remain quiescent until 85% of its volume can be decanted; the clear liquid is siphoned off and replaced by an aqueous solution of sodium chloride (4 g/l) to which is added an antiseptic dilution. This operation is repeated a second time and the vaccine so obtained no longer gives any secondary reaction.

EXAMPLE 7

Improving a pretussis vaccine.

Pertussis vaccines frequently contain, even after washing, bacteria, noxious substances which are badly adsorbed and which, after injection into the organism, can cause secondary reactions. These noxious substances can, moreover, be secreted by the bacteria during ageing of the vaccine. In order to obviate these drawbacks the calcium phosphate gel having adsorbed pertussis vaccine is washed in the following manner.

100 ml of suspension of the adsorbed vaccine are left in a cool chamber for 48 hours. All the clear supernatant liquid is removed and replaced by a saline solution of 9 g NaCl per litre containing a suitable dose of antiseptic. The yellowish supernatant liquid which has been eliminated contained noxious substances; its replacement by the saline solution has rendered the vaccine far more safe and inoffensive.

In another similar preparation the initial suspension was left in the cool chamber for 5 weeks; only then is the above described treatment carried out. In this manner the treatment has not only eliminated the noxious substances which were initially present but

also those which the adsorbed microbes had secreted during the 5 weeks of ageing.

EXAMPLE 8

Four preparations of diphtheria vaccine are made under conditions analogous to those of Example 1, the inactivated polio-virus vaccine solution being replaced by that of diphtheria toxoid. In each case 1 litre of bisodium phosphate solution of 0.07 to 0.0735 M concentration containing the toxoid and 1 litre of 0.07 to 0.0735 M $CaCl_2$ solution were mixed. The latter is poured into the former over a period which differs for each of the operations A to D with stirring.

A	10 seconds
B	10 minutes
C	20 minutes
D	30 minutes

Following precipitation of the calcium phosphate the pH of the aqueous suspension obtained is measured and then brought to the identical value of 6.85 in each of the four cases by addition of 1N NaOH.

The results of determinations of the speed of decantation and of various analyses performed on the suspensions A to D are given below.

TABLE I

Preparation	pH at the end of precipitation	ml of 1N NaOH required per litre of suspension to bring the pH to 6.85
A	5.7	14.8
B	6.5	1.2
C	6.0	7.0
D	6.05	5.2

It is apparent that the suspension obtained by an extremely rapid precipitation (A) has the lowest pH.

After adjustment of the pH the speed of sedimentation is measured on 50 ml of each of the suspension A to D stirred again. These measurements are effected at 20°C in graduated test tubes of 125 mm height (2.5 mm height per ml of capacity). The following levels of clear liquid are found after the interval indicated in table II.

TABLE II

Times	Level in mm of clear liquid as function of time			
	A	B	C	D
5 minutes	2.5	48	48	57
10 minutes	3.8	82	83	89
20 minutes	6.3	92	91	95
1 h 15	21.2	100	95	105
17 hours	85.0	105	105	109

It follows from these measurements that the speed of sedimentation of the suspension (A) of the invention is much slower than that of suspension (B, C, D) prepared in conventional manner, i.e. by progressive addition of $CaCl_2$ to a solution of bisodium phosphate. The sedimentation of the suspension A is roughly 20 times slower than that of suspensions B, C or D during the first 10 minutes.

Other tests have led to the observation that, according to the invention, the gels of calcium phosphate have improved qualities when their aqueous suspensions, substantially 0.035 molar (about 1.4 g Ca/l. and 1.08 g P/l.) clear from 1 to 20 mm, and preferably from 2 to 10 mm, during the first ten minutes at 20°C; vaccines which are particularly well adsorbed and easy to inject are obtained with suspensions of type A clearing no more than 6 mm in 10 minutes.

The sedimentation rates of gel A of Table II have virtually not varied after one or two washings, by decantation, of this gel with a solution of 4 g NaCl per litre water.

Having adsorbed diphtheria toxoid during their above described preparation the suspensions of gels A, B, C, D contain 120 flocculation units (Uf) per ml. Following their sedimentation their activity is determined by the flocculation method of Ramon, on the decanted liquid, which gives the following results:

A	0
B	50 Uf/ml
C	5 Uf/ml
D	7.5 Uf/ml

It can thus be seen that the phosphates B, C and D leave unadsorbed toxoid (5 to 50 Uf/ml) while the gel according to the invention adsorbs it integrally.

On the other hand, washing of the gel A by decantation with an aqueous solution of 4 g NaCl per litre does not cause the appearance of the toxoid in the saline solution, either after one or after two washings.

Analyses of whole suspensions A to D, of the decanted liquids and of the gels themselves have produced the results tabulated in Table III which follows. In this table the

contents of Ca and P are expressed in g/litre; that is to say on the one hand for the stirred, entire suspension as obtained after precipitation and adjustment of the pH to 6.85; on the other hand for the clear liquid separated from the precipitated phosphate by sedimentation; and finally for a suspension brought back to its initial volume by replacing the decanted liquid by distilled water. The Ca/P ratios in Table III are by weight.

TABLE III

		A	B	C	D
Time of $CaCl_2$ addition during precipitation		10 sec	10 min	20 min	30 min
Ca/P ratio of reagents utilised		1.32	1.28	1.28	1.34
Whole initial	Ca	1.409	1.468	1.428	1.438
Suspension	P	1.070	1.150	1.120	1.070
Decanted liquid	Ca	0.026	0.068	0.040	0.046
	P	0.315	0.117	0.197	0.172
Gel separated	Ca	1.383	1.400	1.388	1.392
fom its liquid	P	0.755	1.033	0.923	0.898
and resuspended in water	Ca/P	1.83	1.35	1.51	1.55

These results demonstrate that the very rapid preciptation (A) leads to a liquid medium containing less of Ca and many more phosphorus ions than the liquids of the conventional precipitations (B, C, D), on the other hand the precipitated phosphate is much more rich in calcium: Ca/P ratio = 1.83 against 1.35 to 1.55. Although from the point of view of its composition the phosphate D comes near to the lower limit (Ca/P = 1.55) of that of the invention, it nevertheless differs greatly therefrom in its physico-chemical properties: it has been seen above that its sedimentation rate in 10 minutes is 89 mm, i.e. 23.5 times that of the gel A which is due to a granular structure detrimental to parenteral administration. It has also been seen that the adsorption capability of the phosphate D is poor.

When the gel of phosphate A has been subjected to two washings with 4 g/l. NaCl in the manner described at the beginning of Example 1 and its suspension has been brought back to the initial volume no more than 0.06 g P/litre is found in its clear liquid; the content of Ca therein is 0.03 g/l. The thus lowered concentration of phosphorus ions is no longer inconvenient since the precipitated phosphate has virtually the same composition as prior to the washings; in fact, a weight ratio Ca/P of 1.81 is found, against 1.83 prior to washings. In the aggregate, Ca/P = 1.8 would correspond to $2(Ca_3(PO_4)_2 \cdot CaHPO_4$.

Although the phosphate concentration in the vaccines according to the invention can vary within wide limits it is generally virtually such that there are 1 to 2 g of Ca and 0.56 to 1.48 g of P per litre. However, and this is an advantage of the invention, the vaccine can be preserved in the form of a stable concentrate consisting of a phosphate gel of about 5 to 20 g of Ca and, respectively, about 2.8 to 14.3 g of P per litre.

WHAT WE CLAIM IS:—

1. A process for the preparation of a vaccine adsorbed on a gel of calcium phosphate by contacting an antigen with an aqueous gel obtained by the reaction between bisodium phosphate and calcium chloride, wherein a solution of calcium chloride in water is poured within less than 3 minutes into an equimolar amount of an aqueous solution of stirred bisodium phosphate, and wherein, with continuing stirring, the pH of the mixture is adjusted, after the end of the calcium chloride addition, to a value of 6.8 to 7.2, the gel obtained being subsequently cleansed by sedimentation and decantation.

2. Process according to claim 1, wherein each of the two mixed solutions has a salt content corresponding to 0.01 M to 0.5 M and preferably to 0.05 M to 0.1 M, the respective volumes of these solutions used being such that substantially 1 mol of Na_2HPO_4 is present per 1 mol of $CaCl_2$.

3. Process according to claim 1 or 2, wherein the addition of calcium chloride is effected in an interval of 2 to 30 seconds.

4. Process according to any one of claims 1 to 3, wherein the operation is performed at a temperature of 5° to 40°C.

5. Process according to any one of claims 1 to 4, wherein the reaction mixture is left to stand until 80 to 90% of its total volume has separated as a supernatant layer of clear liquid which is then removed, these standing and removal operations preferably being repeated two to four times.

6. Process according to claim 5, wherein the clear liquid which has been removed is replaced by a solution of NaCl, preferably of concentration 4 to 9 g per litre.

7. Process according to any one of claims 1 to 6, wherein one or a plurality of antigens in aqueous medium are first adsorbed by mixing with the gel of calcium phosphate, the resultant gel is cleansed by sedimentation

separation of the supernatant liquid, and replacement of the latter by a saline solution, once or several times, and in that then one or a plurality of antigens of a different nature, also in aqueous medium, are adsorbed by the same gel which already contains the first antigen or antigens, and the gel so obtained is washed.

8. Process according to Claim 7, wherein the first adsorption takes place by precipitation of calcium phosphate within a solution of bisodium phosphate containing the antigen or antigens.

9. Vaccine adsorbed on calcium phosphate in the form of an aqueous gel, wherein the ratio of the weight of calcium to that of phosphorus combined in the phosphate is from 1.55 to 1.90 and preferably from 1.62 to 1.85.

10. Vaccine according to claim 9, wherein its contents of Ca is 1 to 2 g/l and that of P is 0.56 to 1.48 g/l, respectively.

11. Vaccine according to claim 9 or 10, wherein it contains 5 to 20 g of Ca and 2.8 to 14.3 g of P per litre respectively.

12. Aqueous gel of calcium phosphate wherein the calcium and the phosphorus contained therein are combined in weight ratios Ca/P of 1.55 to 1.90 and preferably of 1.62 to 1.85.

13. Gel according to claim 12, wherein it contains 5 to 20 g of Ca per 2.8 g to 14.3 g of P per litre.

14. Adsorbed vaccine according to claim 10, wherein the level of its liquid which sediments in 10 minutes is from 1 to 20 mm at 20°C and preferably from 2 to 10 mm, for an increased suspension of gel of which the concentration is approximately 0.035 moles of phosphate per litre.

15. Gel according to claim 12, wherein the level of its liquid which sediments in 10 minutes at 20°C is from 1 to 20 mm, for an increased suspension of gel of which the concentration is approximately 0.035 moles of phosphate per litre.

16. A concentrate of vaccine, substantially as described herein.

17. An aqueous gel as claimed in claim 12, substantially as hereinbefore described.

18. A process as claimed in claim 1, substantially as hereinbefore described.

19. A process for the preparation of vaccine, substantially as described in any one of the examples.

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